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Award Number: W81XWH-12-1-0190

TITLE: The Role of Brain Inflammation in Epileptogenesis in TSC

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St Louis, M0
6 3 110
REPORT DATE: J uly 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
J uly 2013	Annual	1 July 2012 – 30 June 2013

4. TITLE AND SUBTITLE
The Role of Brain Inflammation in Epileptogenesis in TSC

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0190

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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Washington University School of Medicine

St. Louis, MO 63110

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Epilepsy is a common, disabling problem in patients with tuberous sclerosis complex (TSC) and is usually intractable to available treatments. This Exploration-Hypothesis Development Award investigates the hypothesis that inflammation contributes to epileptogenesis in an animal model of TSC and that anti-inflammatory drugs may represent rational, effective therapies for epilepsy in TSC. In the first grant year, we have identified a series of inflammatory cytokines and chemokines, such as interferon IL1-beta and CXCL10, based on polymerase chain reaction, western blotting, and immunohistochemistry, which are elevated in a mouse model of TSC. Furthermore, these inflammatory markers could be reversed by the mammalian target of rapamycin (mTOR) inhibitor, rapamycin. These findings suggest that these inflammatory mediators could be involved in epileptogenesis in the mouse model and support the testing of anti-inflammatory agents that inhibit these mediators as potential treatments for epilepsy in the mice.

15. SUBJECT TERMS
Epilepsy, inflammation, tuberous sclerosis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	7	USAMRMC

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Introduction

Epilepsy is a very common, disabling neurological manifestation of Tuberous Sclerosis Complex (TSC), affecting up to 90% of individuals with TSC and causing significant morbidity and mortality [1]. Seizures in TSC patients are often refractory to the available medical and surgical therapies. While much progress has been made in understanding epilepsy in TSC, the specific pathophysiological mechanisms causing epileptogenesis in this disease are still largely unknown. Obtaining a better understanding of these mechanisms of epileptogenesis should lead to more effective therapies for seizures in TSC, including "antiepileptogenic" treatments to prevent epilepsy. Recently, the role of brain inflammation in the pathophysiology of various types of epilepsy has received increasing attention, especially in response to epileptogenic brain injuries [2]. Proinflammatory markers have been found to be activated in a variety of animal models of epilepsy, including chemoconvulsant and electrical kindling models of epilepsy, as well as in human tissue obtained from epilepsy patients, such as with mesial temporal sclerosis. Different types of inflammatory mediators and pathways have been implicated, including cytokines, the complement system, and cyclooxygenase/prostaglandins [2-4]. Furthermore, anti-inflammatory treatments targeting these pathways have begun to be explored. For example, selective pharmacological inhibition of the cytokine IL-1β production in astrocytes blocks kindled seizures in rats [5]. While activation of inflammatory pathways during epileptogenesis in response to acquired brain injury is perhaps not surprising, a relatively novel idea is that brain inflammation could also be important in the pathophysiology of developmental or genetic epilepsies. In fact, many of the inflammatory markers implicated in models of acquired epilepsy due to brain injury have also been found in brain specimens from patients with malformations of cortical development [6,7]. Furthermore, evidence for activation of proinflammatory pathways, such as IL-1β and components of the complement cascade, have been demonstrated in cortical tubers from TSC patients [7-9]. Many of the inflammatory reactions, such as in the cytokine system, appear to be most closely associated with glial cells, including reactive astrocytes and activated microglia.

While these pathological studies from human TSC brain specimens suggest a possible role of inflammatory mechanisms in epileptogenesis, whether inflammation is pathogenic, compensatory, or an epiphenomenon relative to the neurological manifestation of TSC is not known. In this Exploration-Hypothesis Development Award, *we will test the novel hypothesis that inflammation promotes epileptogenesis in TSC*. As glial cells are important mediators of inflammatory reactions, this will be achieved first by examining the expression of different components of inflammatory pathways, including cytokines and prostaglandin systems, in a knock-out mouse model of TSC (*Tsc1*^{GFAP}CKO mice) [10], involving conditional inactivation of the *Tsc1* gene primarily in glia (Task/Aim 1). Then, the effect of anti-inflammatory drugs, inhibiting these specific pathways, will be tested on the development of epilepsy in this mouse model (Task/Aim 2). This project has the potential to reveal novel information about the role of inflammation in epileptogenesis in TSC and to support novel therapeutic approaches involving anti-inflammatory agents for the neurological manifestations of TSC.

Body

Task 1/Specific Aim 1: To determine whether inflammatory pathways, including cytokines (IL-1β, IL-6 and TNF-α) and cyclooxygenase/prostaglandins, are abnormally activated in a mouse model of TSC (months 1-12).

Subtask 1a. Immunocytochemistry studies will assess the expression of IL-1β, IL1-R, IL-6, TNF-α, and Cox-2 in *Tsc1*^{GFAP}CKO and control mice.

Subtask 1b. Western blot studies will assess the expression of IL-1β, TNF-α and Cox-2 in *Tsc1*^{GFAP}CKO and control mice.

Subtask 1c. ELISA studies will assess the expression of IL-1β in *Tsc1*^{GFAP}CKO and control mice.

Over the first year of the grant, we have essentially completed the experiments in Task 1. First, in preparation for this project, we conducted pilot studies using quantitative polymerase chain reaction (q-pcr) to screen a large number of inflammatory markers, including the cytokines and prostaglandins listed above, as well as other related mediators of inflammation, such as chemokines. Four week old control and *Tsc1*^{GFAP}CKO mice were compared, correlating with the age that typically coincides with the onset of epilepsy in the KO mice. mRNA levels of the cytokine IL-1β and IL-6, as well as of the chemokine CXCL10, were upregulated in *Tsc1*^{GFAP}CKO mice, compared with controls. In contrast, IL1-R, TNF-α and Cox-2 mRNA were not significantly different between the KO mice and controls.

For subtask 1a, consistent with the pilot q-pcr studies, immunohistochemical staining showed dramatically increased expression of IL-1β in cortex and hippocampus of *Tsc1*^{GFAP}CKO mice (Fig. 1). Similarly, IL-6 and CXCL10 immunostaining was increased in *Tsc1*^{GFAP}CKO mice, but there was no difference in IL1-R, TNF-α and Cox-2 (data not shown). For subtask 1b, quantitative western blot analysis also confirmed that the protein levels of IL-1β and CXCL10 (Fig. 2), but not TNF-α and Cox-2, were significantly higher in *Tsc1*^{GFAP}CKO mice than that of control mice. In addition, the increased IL-1β and CXCL10 expression was reversed by rapamycin, indicating that the mammalian target of rapamycin (mTOR) signaling pathway is an upstream mediator of this inflammatory response (Fig. 2). Finally, for subtask 1c, ELISA studies also confirmed an increase in IL-1β in the *Tsc1*^{GFAP}CKO mice (5.9 ± 0.2 relative units versus 4.3 ± 0.4 in control mice; $p < 0.05$ by t-test).

Overall, our studies demonstrate that a subset of cytokines and chemokines, but not prostaglandins, is abnormally activated in *Tsc1*^{GFAP}CKO mice, which correlates with the onset of epilepsy in these mice. We hypothesize that these inflammatory mediators may contribute to epileptogenesis and neuropathological abnormalities in *Tsc1*^{GFAP}CKO mice. These results will help in the selection of anti-inflammatory agents targeting specific inflammatory mediators to test for efficacy against epilepsy in Task 2, as described below.

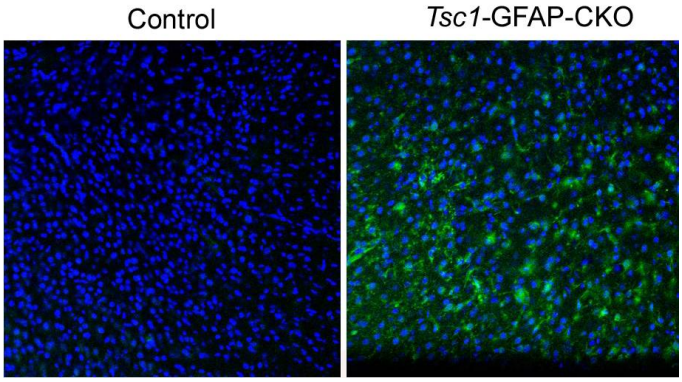

Fig. 1 Increased IL-1β expression in *Tsc1*^{GFAP}CKO mice assayed by immunohistochemistry. Representative images of immunohistochemical staining for IL-1β expression is dramatically increased in neocortex of *Tsc1*^{GFAP}CKO mice (right) compared with control mice (left). **Green: IL-1β**; **Blue: TO-PRO-3 nuclear stain.**

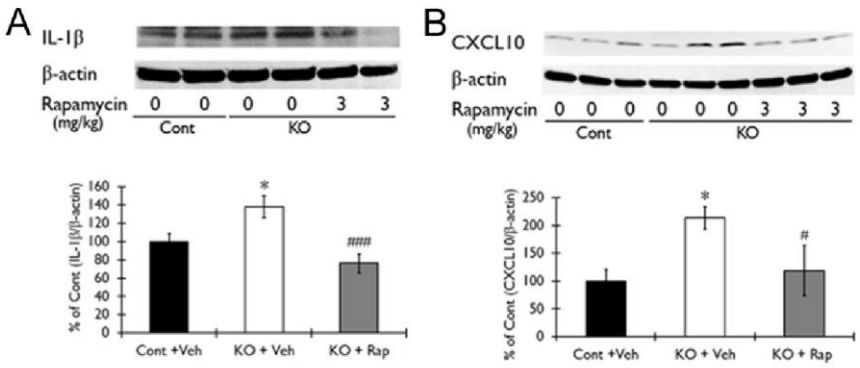

Fig. 2 Increased IL-1β and CXCL10 expression in *Tsc1*^{GFAP}CKO mice assayed by western blotting. *Tsc1*^{GFAP}CKO mice had increased IL-1β (A) and CXCL10 (B) expression in cortical homogenates compared with control mice. Treatment with rapamycin inhibited these increases in IL-1β and CXCL10 in *Tsc1*^{GFAP}CKO mice.

Task 2/Specific Aim 2: To determine whether anti-inflammatory agents targeting cytokine or prostaglandin production prevent epilepsy in a mouse model of TSC (months 13-24).

Subtask 2a. Immunocytochemistry and western blot studies will be performed to assess the effect of cytokine inhibitors and cyclooxygenase inhibitors on expression of cytokine or Cox-2 in *Tsc1*^{GFAP}CKO mice (months 13-15; ~30 mice total).

Subtask 2b. Histological studies will be performed to determine the effect of cytokine inhibitors and cyclooxygenase inhibitors on histological abnormalities of *Tsc1*^{GFAP}CKO and mice (months 16-18; ~30 mice total).

Subtask 2c. Video-EEG studies will be performed to determine the effect of cytokine inhibitors and cyclooxygenase inhibitors on the development of epilepsy and interictal EEG abnormalities in *Tsc1*^{GFAP}CKO mice (months 19-24; ~30 mice total).

Task 2 will be completed in the second year of the grant. Given the results of Task 1 above, we will focus primarily on specific cytokine and chemokine inhibitors targeting IL-1β, IL6, and CXCL10, respectively.

Key Research Accomplishments

In the first year of this grant, we have obtained the following experimental results related to Task 1:

- Quantitative pcr demonstrates that mRNA expression of the cytokines IL-1β and IL-6 and the chemokine CXCL10 is elevated in *Tsc1*^{GFAP}CKO mice, compared with controls. In contrast, IL1-R, TNF-α and Cox-2 mRNA expression is unchanged.
- Immunohistochemical studies show increased IL-1β, IL-6 and CXCL10 immunostaining in neocortex and hippocampus of *Tsc1*^{GFAP}CKO mice, but there was no difference in IL1-R, TNF-α and Cox-2.
- Quantitative western blot analysis also confirmed that the protein levels of IL-1β and CXCL10, but not TNF-α and Cox-2, were significantly higher in *Tsc1*^{GFAP}CKO mice than that of control mice.
- The increased IL-1β and CXCL10 expression was reversed by rapamycin, indicating that the mammalian target of rapamycin (mTOR) signaling pathway is an upstream mediator of this inflammatory response.
- ELISA studies also confirmed an increase in IL-1β in the *Tsc1*^{GFAP}CKO mice.

Reportable Outcomes

None to date.

Conclusion

In the first year of this grant, we have demonstrated that specific inflammatory cytokines and chemokines are abnormally activated during epileptogenesis in *Tsc1*^{GFAP}CKO mice. In contrast, prostaglandins, at least as assayed by Cox-2 expression, did not appear to be similarly involved. Consistent with our primary hypothesis, these results suggest that these inflammatory mediators could be involved in epileptogenesis in *Tsc1*^{GFAP}CKO mice and identify specific mechanistic targets in inflammatory pathways as potential treatments for preventing epilepsy (to be tested in Task 2). Ultimately, the findings from this grant may implicate a novel role of inflammation in epilepsy in TSC and support the use of anti-inflammatory agents as antiepileptogenic therapies for TSC patients.

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Appendices

None